Structure and Composition of Mushrooms as Affected by Hydrogen Peroxide Wash

G.M. Sapers, R.L. Miller, S.-W. Choi, and P.H. Cooke

ABSTRACT

An experimental H_2O_2/browning inhibitor wash treatment and its effect on mushroom structure and composition were studied. Experimentally washed mushrooms (*Agaricus bisporus*) were compared with conventionally washed mushrooms and untreated controls. Examination by scanning electron microscopy showed damage to hyphae producing a matted appearance at the pileus surface with both experimental and conventional washed samples. Mushrooms after the experimental wash had an elevated sodium content from the sodium erythorbate browning inhibitor but contained no H_2O_2 residue. In pileus tissue, soluble phenol levels were higher and the content of free amino acids was lower in mushrooms after the experimental wash. No other notable compositional differences or adverse effects of treatment on quality were found.

Key Words: mushrooms, hydrogen peroxide, washing process, composition, structure

INTRODUCTION

FRESH MUSHROOMS FREQUENTLY CONTAIN FRAGMENTS OF SOIL either embedded in the surface or as loose particles in the container, which detract from product appearance. As a result, they require cleaning before consumption as condiments for salad bars or pizza toppings or in other food service applications. Attempts to wash mushrooms, with or without antimicrobial agents such as bisulfite or chlorine to extend shelf-life, generally have not been successful, due to rapid product spoilage (Guthrie and Beelman, 1989). We developed an experimental washing system that used hydrogen peroxide (H_2O_2) as an antimicrobial agent and did not result in premature spoilage (Sapers and Simmons, 1998). However, the treatment would induce browning unless a browning inhibitor such as sodium erythorbate could be applied following exposure of mushrooms to H_2O_2 (Sapers et al., 1994, 1995a). With our experimental washing system, a shelf life of at least 1 wk could be realized compared to 4 to 6 days for conventionally washed mushrooms (Sapers et al., 1995a, 1995b). Our objective was to analyze mushrooms after the experimental treatment, a conventional wash with water, or no treatment to help identify physical or chemical changes that might affect regulatory approval of the experimental treatment.

MATERIALS & METHODS

Sample preparation

Mushrooms for analytical studies were prepared from first quality raw material in a commercial packing plant by the investigators and the cooperating packer with a continuous system for the conventional wash and a batch system for the experimental wash (Sapers et al., 1995a). Water for the conventional wash contained 2 to 5 ppm chlorine (as calcium hypochlorite). The experimental system comprised a wash with 5% H_2O_2 and a spray application of 4% sodium erythorbate solution. The drained product was packaged in perforated polyvinyl chloride film, vacuum cooled, and transported to our laboratory over ice in insulated coolers for further preparation and analysis.

Scanning electron microscopy

For scanning electron microscopy (SEM), freshly prepared mushroom samples were fixed with 1% glutaraldehyde, and excised tissue was dehydrated with ethanol, critical point dried and sputter coated with gold (Sapers et al., 1994). A JEOL SEM (Model 840 A) was operated in the secondary electron imaging mode.

Determination of residual H_2O_2 in treated mushrooms

Peroxide residues were determined by two qualitative procedures in samples given the above treatment but prepared in our laboratories. The determinations were carried out within a few minutes of mushroom exposure to the H_2O_2 solution. Treated mushrooms were blended with an equal weight of water and the homogenate tested for H_2O_2 with an EM Quant Peroxide Test Strip (EM Science, EM Industries Inc., Gibbstown, N.J., U.S.A., sensitivity 0.5 ppm). Alternatively, 71.5 g treated mushrooms were thoroughly rinsed with 200 mL water to remove residual H_2O_2 and an aliquot of the rinse water was tested by the TOOS-4AA qualitative color test of Miyamoto et al. (1993) (sensitivity 0.1 ppm H_2O_2).

Phenolic compounds and free amino acids

Phenolic compounds and free amino acids were determined on mushrooms given the experimental wash, conventionally washed mushrooms, and untreated controls from the commercial packer. Samples were frozen at −18 °C, and lyophilized to constant weight in a Model 5SL freeze drier (The Virtis Co., Gardiner, N.Y., U.S.A.). Three subsamples of each treatment, designated I, II, and III, were shipped to the Korean investigator by express mail. The lyophilized mushrooms were dissected into specific tissues with a spatula. Soluble phenols were extracted by shaking in hot methanol in a water bath at 80 °C for 2.5 h. After filtering, soluble phenol levels were determined in triplicate by the Folin-Ciocalteu method described by Paranjpe et al. (1978). Gallic acid was used as the standard for the soluble phenol determination.

Analyses were performed for 2 important compounds found in mushrooms: λ-L-glutaminyl-4-hydroxybenzene (GHB) and tyrosine. HPLC was used to separate and quantify these compounds, as described by Choi and Sapers (1994). Determinations were made in triplicate.

Free amino acids in lyophilized mushroom samples were determined in duplicate by extraction with 0.02 N HCl-MeOH (30:70) containing 0.02% dithiothreitol. Extracts were partially purified with a Sep-Pak cartridge, and the free amino acids were separated with an automated amino acid analyzer (Pharmacia Biotech), using a SCX1001 resin column eluted with lithium citrate buffer (pH increased from 2.2 to 3.6).

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Proximate and other analyses

Proximate analyses and analyses for specific mushroom constituents were performed in duplicate (designated A and B) on composite samples of each treatment by Lancaster Laboratories (Lancaster, Pa., U.S.A.) using AOAC procedures (AOAC, 1990), except where indicated otherwise: moisture (926.08, 925.45, 934.06, 969.38, 925.40, 926.12), protein (990.03, 992.23), fat (945.44), total dietary fiber (AOAC, 16th ed; 991.43), ash (AOAC, 16th ed.; 968.08, 969.23), mannitol (Marowski, 1992), total tyrosine (982.30), total lysine (982.30), cis-mono-unsaturated fatty acid (Sheppard, 1992), linoleic acid (AOCS, 1993; Ce 1c-89), linolenic acid (AOCS 1c-89), vitamin C (967.22), riboflavin (981.15), niacin (981.16), sodium (AOAC, 16th ed; 968.08, 969.23).

Informal quality evaluation

An untrained panel comprising 2 of the investigators and 2 to 3 experienced employees of the cooperating mushroom packer evaluated replicate samples at our Center and at the packing house after storage at 3 to 4 °C for 3 to 10 days. Samples were informally examined visually, and after some trials, tasted in the raw state.

Statistical analysis

Composition data were analyzed for differences in response to treatment by ANOVA and the Bonferroni LSD test to separate means (Miller, 1981). The amino acid data were analyzed by calculating relative differences between treatment means for experimental and conventional treatments and performing paired t-tests on these relative differences; pileus and stipe data were analyzed separately. All statistical analyses were performed with the SAS/STAT software (SAS Institute, Inc., Cary, N.C., U.S.A.). Significance of differences was defined at $P \leq 0.05$.

RESULTS & DISCUSSION

Structural changes resulting from treatment

SEM images (100×) of mushroom pileus (cap) surfaces showed the control surface was a relatively open structure of intertwined, intact hyphae (Fig. 1). With conventionally washed mushrooms, hyphae on the pileus surface were severely matted and broken (Fig. 2). This was probably due to mechanical damage during handling, which caused the hyphae to fracture and leak cellular fluids that held them

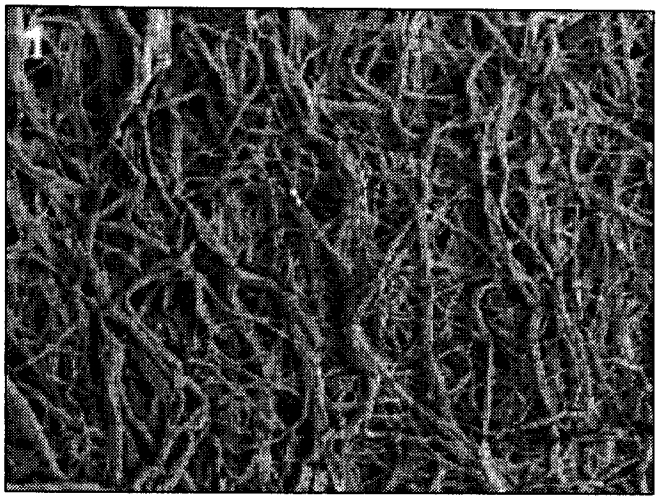

Fig. 1—Scanning electron micrograph of pileus surface of control mushrooms at 100× magnification.

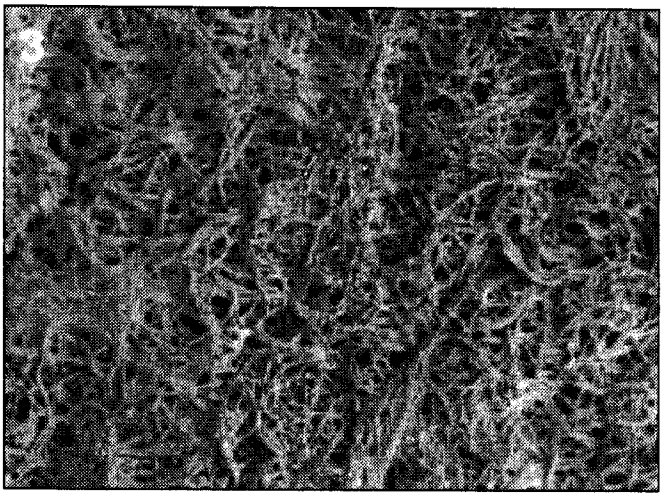

Fig. 3—Scanning electron micrograph of pileus surface of conventionally washed mushrooms at 100× magnification, showing areas of less severe matting.

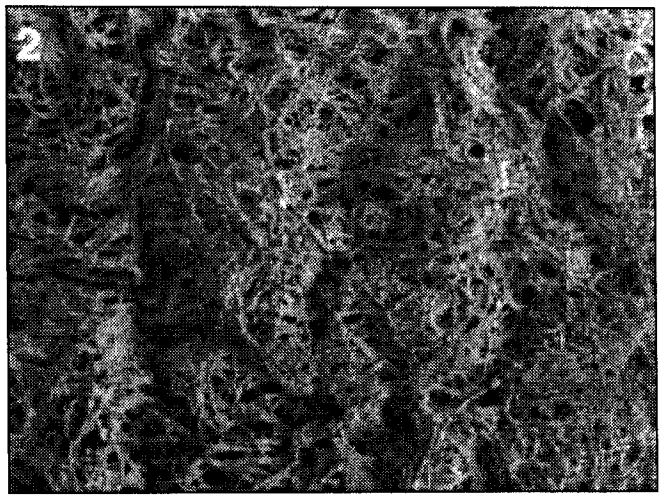

Fig. 2—Scanning electron micrograph of pileus surface of conventionally washed mushrooms at 100× magnification, showing areas of severe matting.

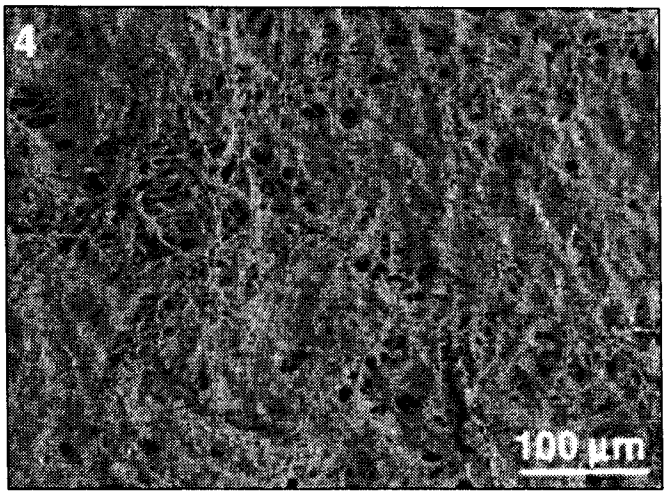

Fig. 4—Scanning electron micrograph of pileus surface of experimentally washed mushrooms at 100× magnification.

together in a mat. Portions of the surface were less extensively matted (Fig. 3). The experimental wash treatment resulted in similar but more extensive hyphae breakage and surface matting than with the conventional wash (Fig. 4). This was probably due to mechanical injury from additional handling during batch washing and spraying with the browning inhibitor, treatments not used with the conventional washing system. Mechanical damage to hyphae in conventionally washed mushrooms predisposes them to rapid spoilage by bacteria that grow in the nutrient-rich exudate from fractured hyphae. This was not the case with mushrooms given the experimental wash treatment as 5% H_2O_2 is lethal to bacteria, reducing the microbial load (Sapers, 1996). Both experimentally and conventionally washed mushrooms showed loss in brightness compared to unwashed controls. This was probably the result of the hyphal damage and matting.

Residual H_2O_2 in treated mushrooms

The *Code of Federal Regulations* (21 CFR 184.1366) specifies that H_2O_2 is permitted for use as an antimicrobial agent at maximum levels of 0.05% to 0.15%, depending on application, provided that "residual hydrogen peroxide is removed by appropriate physical and chemical means during processing." No H_2O_2 residue was detected by the EM Quant Peroxide Test Strip in 3 successive mushroom washing trials with 5% H_2O_2. Similarly, no H_2O_2 was detected by the more sensitive TOOS-4AA procedure in the water used to wash residual H_2O_2 from treated mushrooms. In that procedure, blending was avoided to minimize loss of residual H_2O_2 by reaction with endogenous catalase released by disruption of the mushroom. The absence of H_2O_2 residues in treated mushrooms was expected due to high levels of endogenous catalase in mushrooms. (Copious amounts of O_2 gas are generated during the washing treatment.) Residual H_2O_2 also would be reduced by reaction with erythorbate that had been applied within a few seconds of the H_2O_2 wash.

Phenolic compounds

The experimental wash resulted in greater retention of soluble phenols, both in the pileus and stipe, than the conventional wash or control (Table 1). This may have been a consequence of the sodium erythorbate spray application following treatment with H_2O_2. Erythorbate is an antioxidant and could prevent the loss of phenolic

Table 1—Soluble phenols in pileus and stipe of washed and control mushrooms

		Soluble phenols (mg/g dry weight)			
		Sample[a]			
Treatment	Tissue	I	II	III	Mean[b]
Experimental wash	Pileus	4.75 ± 0.69	—	4.70 ± 0.10	4.72c
	Stipe	4.03 ± 0.37	3.94 ± 0.29	3.42 ± 0.09	3.79C
Conventional wash	Pileus	3.10 ± 0.38	2.87 ± 0.32	3.08 ± 0.13	3.02d
	Stipe	2.40 ± 0.03	2.61 ± 0.09	2.56 ± 0.08	2.52D
Control	Pileus	3.03 ± 0.25	2.83 ± 0.17	2.92 ± 0.10	2.93d
	Stipe	3.07 ± 0.25	3.35 ± 0.24	2.75 ± 0.12	3.06D

[a]Mean of triplicate determinations ± standard deviation.
[b]For a given tissue, means with no letter in common are different (p<0.05) by the Bonferroni LSD method; small letters refer to pileus tissue, and capital letters stipe tissue.

Table 2—GHB in pileus and stipe of washed and control mushrooms

		GHB content (μM/g dry weight)			
		Sample[a]			
Treatment	Tissue	I	II	III	Mean[b]
Experimental wash	Pileus	13.75 ± 0.19	12.82 ± 0.21	12.01 ± 0.10	12.85d
	Stipe	48.23 ± 0.34	42.73 ± 0.30	41.92 ± 0.20	44.29C
Conventional wash	Pileus	14.25 ± 0.22	13.82 ± 0.21	12.11 ± 0.20	13.39c
	Stipe	41.61 ± 0.24	41.11 ± 0.16	42.23 ± 0.20	41.65C
Control	Pileus	15.26 ± 0.28	15.01 ± 0.20	13.91 ± 0.14	14.73c
	Stipe	42.31 ± 0.32	43.10 ± 0.20	41.12 ± 0.14	42.18C

[a]Mean of triplicate determinations ± standard deviation.
[b]For a given tissue, means with no letter in common are different (p<0.05) by the Bonferroni LSD method; small letters refer to pileus tissue, and capital letters stipe tissue.

Table 3—Tyrosine in pileus and stipe of washed and control mushrooms

		Tyrosine content (μM/g dry weight)			
		Sample[a]			
Treatment	Tissue	I	II	III	Mean[b]
Experimental wash	Pileus	2.88 ± 0.32	2.99 ± 0.30	3.01 ± 0.22	2.96cd
	Stipe	2.11 ± 0.34	2.09 ± 0.30	2.14 ± 0.20	2.11C
Conventional wash	Pileus	2.79 ± 0.30	2.42 ± 0.20	2.58 ± 0.19	2.60d
	Stipe	2.18 ± 0.20	2.16 ± 0.22	2.21 ± 0.21	2.18C
Control	Pileus	3.19 ± 0.12	3.21 ± 0.22	3.31 ± 0.22	3.24c
	Stipe	2.26 ± 0.21	2.19 ± 0.18	2.45 ± 0.18	2.30C

[a]Mean of triplicate determinations ± standard deviation.
[b]For a given tissue, means with no letter in common are different (p<0.05) by the Bonferroni LSD method; small letters refer to pileus tissue, and capital letters stipe tissue.

Table 4—Free amino acid content of washed and control mushrooms[a]

	Free amino acid content (μM/g dry weight)[b]					
	Experimental wash		Conventional wash		Control	
Amino acid	Pileus	Stipe	Pileus	Stipe	Pileus	Stipe
Phosphoserine	4.6±2.8	13.8±8.2	10.2±6.2	6.6±3.7	8.3±5.5	7.6±4.4
Aspartic acid	60.6±34.8	24.7±12.1	85.0±32.2	23.1±9.2	81.7±34.3	32.4±10.2
Threonine	34.8±10.2	13.8±4.9	57.2±11.5	15.1±6.2	50.2±21.1	18.8±5.0
Serine	50.6±18.2	34.6±19.3	72.6±18.2	30.7±11.5	64.3±20.5	40.6±11.3
Asparagine	94.1±36.2	34.8±19.2	139.1±64.2	40.6±11.2	140.4±71.6	55.4±26.2
Glutamic acid	170.6±81.4	110.8±77.1	272.8±121.2	120.4±69.4	224.8±102.1	146.9±91.2
Proline	74.2±18.8	86.0±17.2	68.0±19.5	95.2±30.7	57.7±14.7	88.3±20.7
Glycine	41.8±11.6	19.8±5.7	58.2±17.4	23.2±5.2	48.0±20.8	24.6±5.4
Alanine	338.4±150.8	177.8±72.3	540.4±290.3	195.2±98.6	352.2±178.9	228.2±101.7
Valine	28.2±5.8	9.0±2.4	42.8±18.4	13.4±2.9	34.4±7.7	13.3±3.3
Cystine	6.2±1.5	2.9±0.5	12.8±3.2	4.7±1.1	8.0±2.8	3.8±1.0
Methionine	4.7±1.3	2.9±0.8	6.7±1.3	3.4±1.7	5.6±1.6	3.8±0.6
Cystathionine	6.0±0.9	2.0±0.3	9.4±1.8	2.5±0.3	8.2±1.4	2.8±0.3
Isoleucine	16.6±2.4	6.6±0.9	23.9±5.9	8.4±1.6	20.2±4.0	8.7±1.7
Leucine	25.6±5.0	8.4±4.9	42.4±11.4	11.0±9.0	34.4±9.3	11.0±9.7
β-Alanine	20.4±12.4	46.2±27.4	24.2±9.7	38.2±19.5	23.9±8.1	37.8±10.5
Phenylalanine	16.6±9.5	6.0±3.9	30.8±19.4	8.4±5.2	25.5±11.6	7.7±5.4
-aminoisobutyric acid	21.6±7.2	22.8±5.7	38.1±6.3	29.9±4.4	21.8±4.5	26.1±6.2
Ornithine	76.2±32.2	104.0±42.9	96.6±31.4	99.8±24.3	88.9±34.6	105.8±48.3
Lysine	19.4±6.8	4.8±2.7	23.7±4.0	4.6±1.5	24.6±4.8	6.2±1.0
Histidine	6.7±4.0	3.0±1.4	10.6±5.9	2.0±1.2	9.8±3.4	2.1±1.4
Arginine	4.0±3.2	4.9±3.0	2.6±1.2	2.8±1.1	3.2±2.0	2.3±1.9

[a]From subsample III.
[b]Means for a given tissue are not different (p > 0.05) by the Bonferroni LSD method. Pileus means for experimental and conventional washes were different (paired t-tests, p<0.01).

Table 5—Proximate analysis and other constituents of washed and control mushrooms[a]

Analysis	Experimental wash	Conventional wash	Control
Moisture (Vac. Oven), %[b]	93.0±0.1	93.5±0.0	92.8±0.1
Protein (Mod. Dumas), %	2.2±0.1	2.2±0.1	2.5±0.3
Fat (Acid Hydrolysis), %	0.2±0.1	0.2±0.1	0.15±0.1
Dietary Fiber, Total, %	1.2±0.1	1.0±0.0	0.95±0.1
Ash, %	0.77±0.03	0.75±0.02	0.78±0.01
Mannitol, %	1.64±0.05	1.34±0.01	1.49±0.01
Total Tyrosine, %	0.035±0.01	0.02±0.00	0.025±0.01
Total Lysine, %	0.10±0.01	0.09±0.00	0.11±0.00
Cis-Mono-unsaturated Fatty Acid, %	<0.1	<0.1	<0.1
Linoleic Acid, %	0.13±0.00	0.14±0.02	0.14±0.02
Linolenic Acid, %	<0.03	<0.03	<0.03
Vitamin C, mg/100g	<1.0	<1.0	<1.0
Riboflavin, mg/100g	0.36±0.03	0.31±0.00	0.38±0.01
Niacin, mg/100g	2.5±0.0	2.2±0.1	2.5±0.0
Sodium, mg/100g[c]	26.8±0.5	8.36±0.15	3.36±0.1

[a]Mean of duplicate determinations ± standard deviation.
[b]Percent by weight.
[c]Treatments different from each other ($p<0.05$) by the Bonferroni LSD method.

compounds due to oxidation by H_2O_2 and polyphenol oxidase. The disappearance of phenolics might correlate with an increase in enzymatic browning, but the extent of browning was not measured.

Levels of GHB (Table 2) and tyrosine (Table 3) were generally similar in mushrooms given the three treatments. Pileus tissue contained more tyrosine and less GHB than did stipe tissue.

Free amino acids

Experimentally washed mushrooms showed a reduction ($t = 2.91$, $p \leq 0.01$) in pileus free amino acid content, compared to conventionally washed mushrooms (Table 4). One possible explanation for the lower free amino acid content in the experimental mushroom samples may be leaching during washing and erythorbate application. Fresh mushrooms are not a major dietary component, and the free amino acid losses were generally small (mean loss =19%), so the losses would not likely have any nutritional importance. There were no indications that losses of individual free amino acids were associated with any quality defects.

Proximate and other analyses

Proximate analysis and other analyses for major mushroom constituents indicated no differences between mushrooms given the experimental wash and the other samples (Table 5). The higher sodium content is a reflection of the sodium erythorbate applied to the experimental wash samples.

Mushroom quality and shelf-life

In informal tests mushrooms given the experimental wash showed no evidence of changes in flavor, texture or color after treatment. During storage, experimentally washed mushrooms retained their fresh appearance for as long as 7 to 10 days at 3 to 4 °C while conventionally washed mushrooms showed bacterial blotch within 4 to 6 days, depending on raw material quality. Unwashed controls showed more browning in 7 to 10 days than experimentally washed mushrooms and occasional "ginger" blotch, a yellow-brown discoloration produced by *Pseudomonas gingeri* (Wong et al., 1982; Wells et al., 1996).

The dilute H_2O_2 was very effective in reducing the bacterial population in wash water. Smaller reductions in bacterial load were found in the experimentally washed mushrooms (Sapers and Simmons, 1998).

CONCLUSIONS

AN EXPERIMENTAL WASHING PROCEDURE LEFT NO H_2O_2 RESIDUE on treated mushrooms and only traces of sodium originating from a browning inhibitor. It had minimal effects on mushroom phenolic compounds and free amino acids. SEM indicated that the experimental wash had no effect on mushroom structure other than that attributable to the mechanical effects of washing. Results indicate that the experimental process would have no significant deleterious effects on composition or nutrient content of mushrooms.

REFERENCES

AOAC. 1990. *Official Methods of Analysis,* 15th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC. 1997. *Official Methods of Analysis,* 16th ed. Association of Official Analytical Chemists International, Gaithersburg, MD.

AOCS. 1993. *Official Methods and Recommended Practices of the American Oil Chemists Society*, method Ce 1c-89. Amer. Oil Chem. Soc., Champaign, IL.

21CFR. Code of Federal Regulations 21, Parts 170-199, Section 184.1366, April 1, 1992. Government Printing Office.

Choi, S.-W. and Sapers, G.M. 1994. Effects of washing on polyphenols and polyphenol oxidase in commercial mushrooms (*Agaricus bisporus*). J. Agric. Food Chem. 42: 2286-2290.

Guthrie, B.D. and Beelman, R.B. 1989. Control of bacterial deterioration in fresh washed mushrooms. Mushroom Science XII (Part II): 689-700. *Proceedings of the Twelfth International Congress on the Science and Cultivation of Edible Fungi, Braunschweig, FRG, 1987.*

Marowski, J. 1992. Carbohydrate nutritional labeling. PittCon Paper. Millipore, Waters Chromatography Division, Industrial Lab Marketing Department.

Miller, R.G., Jr. 1981. In *Simultaneous Statistical Inference*, p. 67. Springer-Verlag, New York.

Miyamoto, F., Saeki, M., and Yoshizawa, T. 1993. A sensitive qualitative color test for residual hydrogen peroxide in foods. Jpn. J. Toxicol. Environ. Health 39: 336-344.

Paranjpe, M.S., Chen, P.K., and Jong, S.C. 1978. Phenolic and other organic compounds in morphologically different tissues of *Agaricus bisporus*. Trans. Mycol. Soc. Japan 19: 169-180.

Sapers, G.M., Miller, R.L., Miller, F.C., Cooke, P.H., and Choi, S.-W. 1994. Enzymatic browning control in minimally processed mushrooms. J. Food Sci. 59: 1042-1047.

Sapers, G.M. 1996. Hydrogen peroxide as an alternative to chlorine. Presented at the 1996 Institute of Food Technologists Annual Meeting, New Orleans, LA, Abstract 59-4.

Sapers, G.M., Miller, R.L., and Choi, S.-W. 1995a. Mushroom discoloration: new processes for improving shelf life & appearance. Mushroom News 43(3): 7-13.

Sapers, G.M., Miller, R.L., and Choi, S.-W. 1995b. Prevention of enzymatic browning in prepeeled potatoes and minimally processed mushrooms. Ch. 18 in *Enzymatic Browning and its Prevention*, C.Y. Lee and J.R. Whitaker (Ed.), ACS Symposium Series 600, American Chemical Society, Washington, DC.

Sapers, G.M. and Simmons, G.F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. Food Technol. 52(2): 48-52.

Sheppard, A.J. 1992. Methodology appropriate for fatty acid cholesterol analysis. In *Lipid Manual*, USFDA, Wm. C. Brown, Publ.

Wells, J.M., Sapers, G.M., Fett, W.F., Butterfield, J.E., Jones, J.B., Bouzar, H., and Miller, F.C. 1996. Postharvest discoloration of the cultivated mushroom *Agaricus bisperus* caused by *Pseudomonas tolaasii, P. reactans*, and *P. gingeri*. Phytopathology 86: 1098-1103.

Wong, W.C., Fletcher, J.T., Unsworth, B.A., and Preece, T.F. 1982. A note on ginger blotch, a new bacterial disease of the cultivated mushroom, *Agaricus bisporus*. J. Applied Bacterial. 52: 43-48.

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